Repeated exposure to low levels of the chemical warfare agent VX activates cell survival related gene in mouse brain

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Division of Neuroscience
WRAIR

Report Documentation Page

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Introduction

- VX is an excitotoxic compound.
- Neurons may respond to toxicity by expressing genes associated with cell survival.
- Neurotrophins:
  - neuronal development
  - neuronal plasticity and remodeling
  - cell survival
- Brain derived neurotrophic factor (BDNF) is modulated:
  - traumatic brain injury
  - ischemia
  - toxin exposure
- BDNF may be important for neuronal plasticity, cell survival and remodeling following brain trauma.

Purpose

- Evaluate whether repeated exposure to low levels of VX affects genes associated with cell survival and neuronal plasticity.

Experimental Design

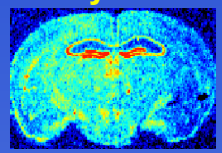
Exposed Animals

Blood levels of AChE and BChE

Brain extraction
(Regions: Ex:Hippocampus)

From Amaral and Witter, 1995

In Situ Hybridization



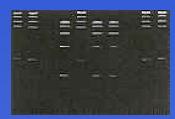
Analysis
(BIO-RAD GS-800)
Calibrated with an Optical Density Tablet

Proteins

mRNA

Gel Electrophoresis

Northern Blots
RT-PCR



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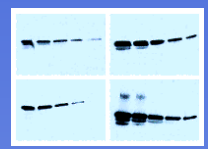

Western Transfer

Animal Exposure

- Repeated low-level exposure
  - Three groups:
    - Saline controls
    - 0.2 LD_{50}
    - 0.4 LD_{50}
  - 1/day for 5 days/wk for 2 wk
- The LD_{50} value for VX given subcutaneously (s.c.) in mice was 21 μg/kg (Boskovic, 1979).
- The nerve agent VX was dissolved in saline and injected s.c. in volume of 1 ml/kg.

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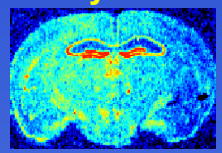
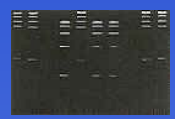


Northern Blots
RT-PCR



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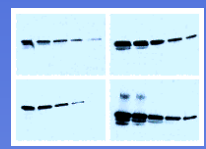
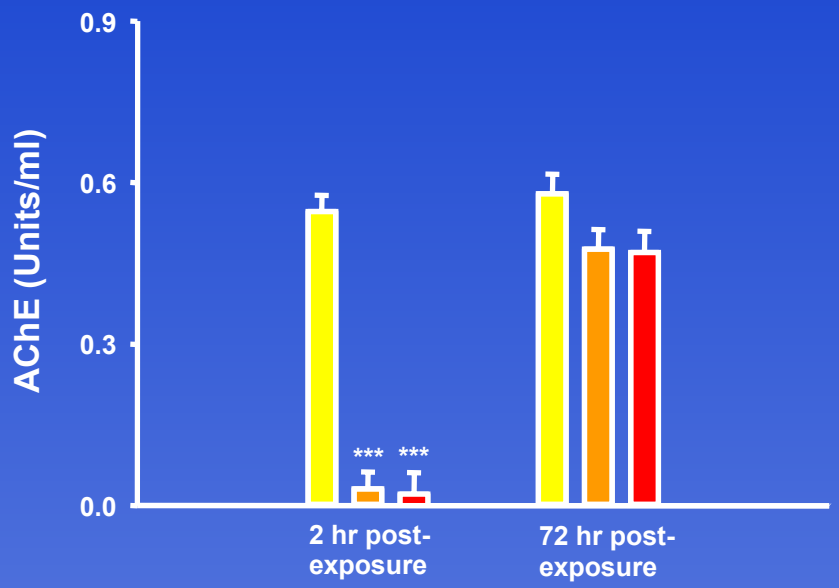
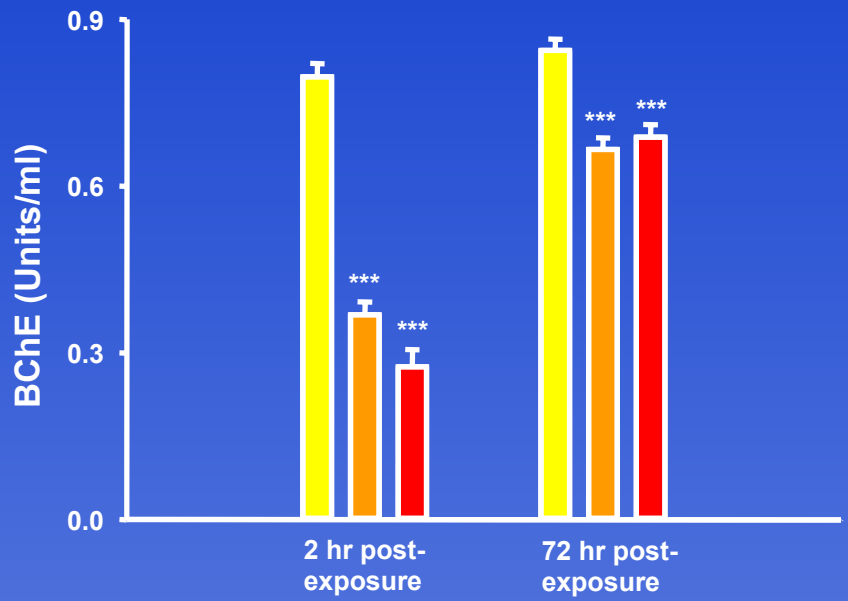


Western Transfer

Blood levels of acetylcholinesterase (AChE)



Blood levels of butyrylcholinesterase (BChE)



Saline (n = 5)

0.2 LD_{50} VX (n = 5)

0.4 LD_{50} VX (n = 6)

Experimental Design

Exposed Animals

Brain extraction
(Regions: Ex:Hippocampus)

From Amaral and Witter, 1995

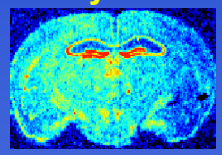
Proteins

mRNA

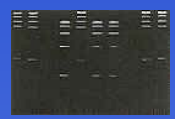
Gel Electrophoresis

Blood levels of
AChE and BChE

In Situ Hybridization



Northern Blots
RT-PCR



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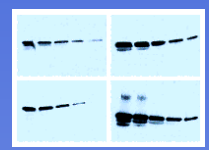

Western Transfer

In situ Hybridization

- RNA probes for BDNF were generated by *in vitro* transcription using Strip-EZ™ RNA.
- For antisense ^{33}P-UTP labeled BDNF RNA probes, a plasmid containing a 460 bp BDNF
  - linearized with EcoRI
  - transcribed with T7 polymerase
- For sense ^{33}P-UTP labeled BDNF RNA probes
  - linearized with SalI
  - transcribed with SP6 polymerase

In situ Hybridization (cont.)

- Coronal sections (14 μm) were hybridized overnight with ^{33}P-UTP labeled cRNA probe.
- The sections were then exposed to a series of high temperature washes in various dilutions of SSC.
- The sections were then air-dried and autoradiographed on Kodak BioMax MS.
- The film was analyzed using the Model GS-800 Calibrated Imaging Densitometer and the Quantity One software.

Experimental Design

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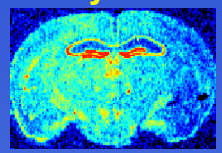
In Situ Hybridization



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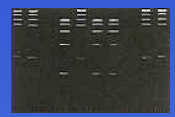

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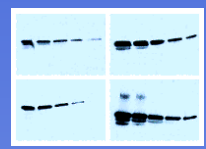
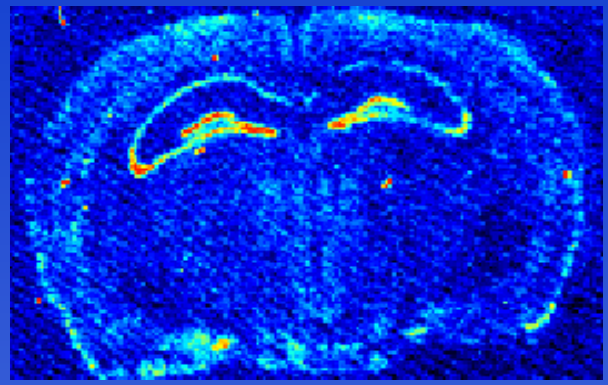
Immunostaining



Western Transfer

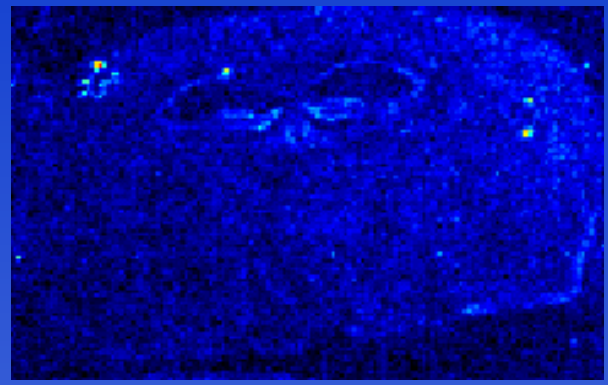
In situ hybridization cRNA controls

A



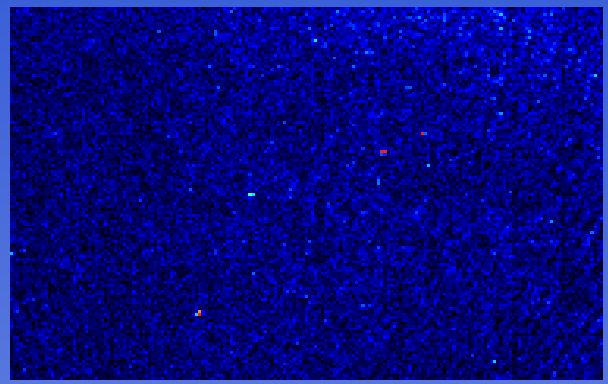
Control

B



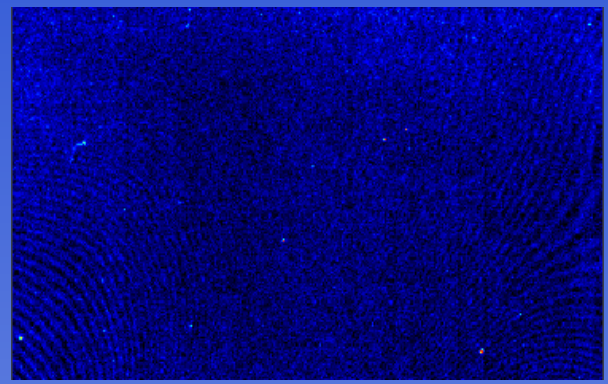
Sense

C



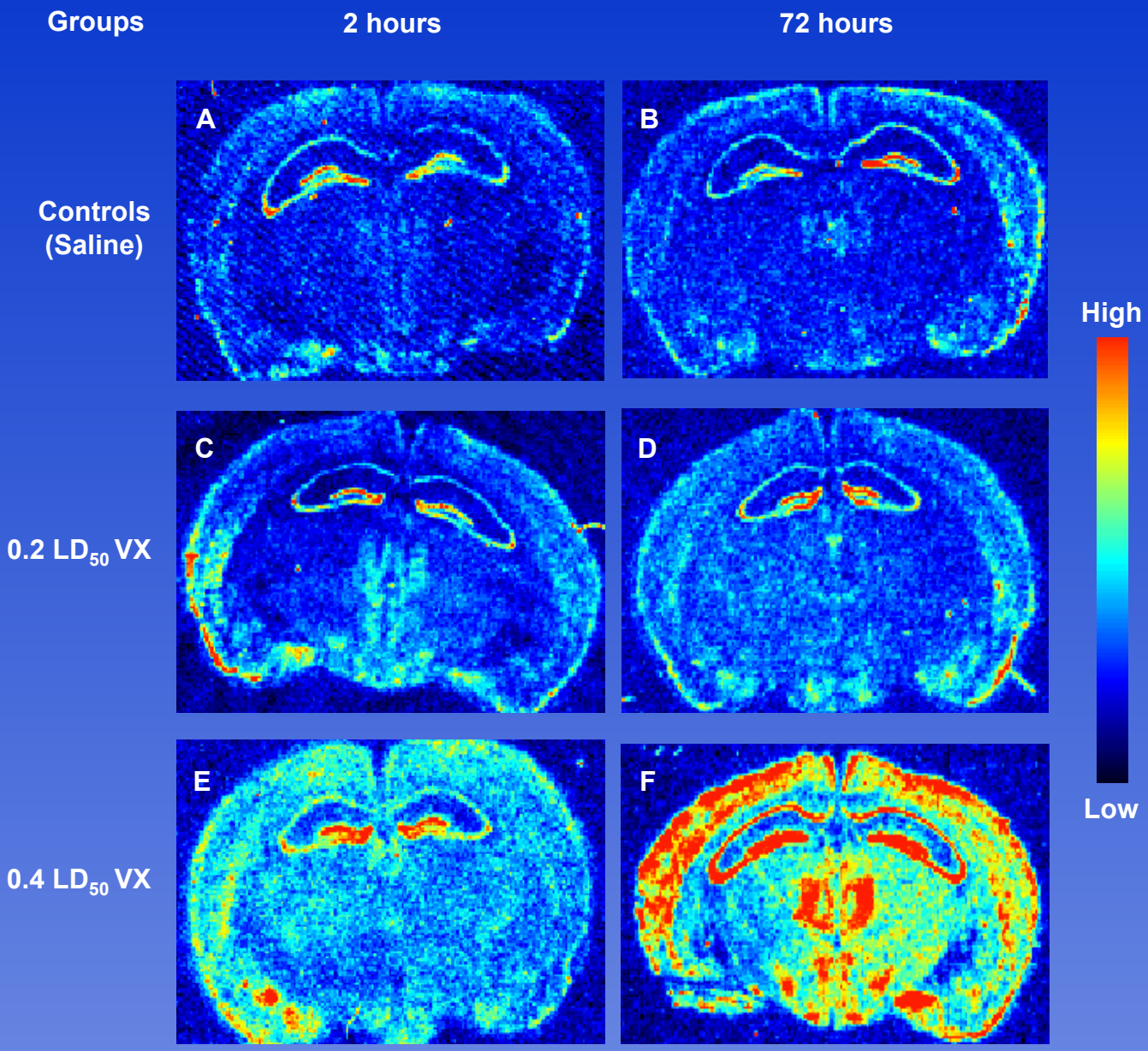
Blocked

D

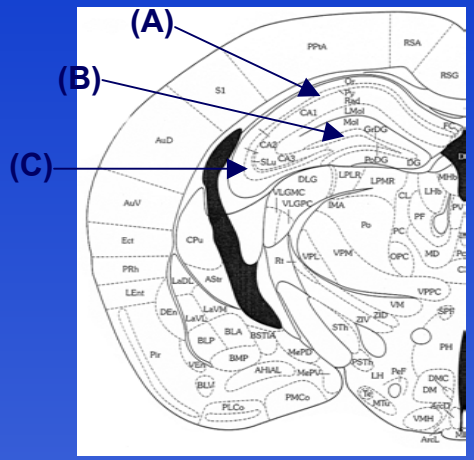


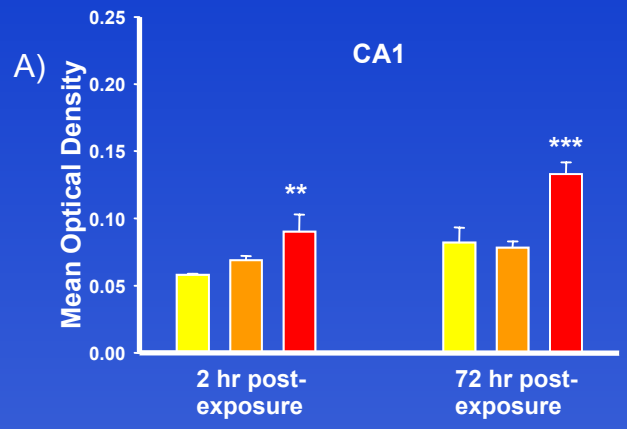
RNase

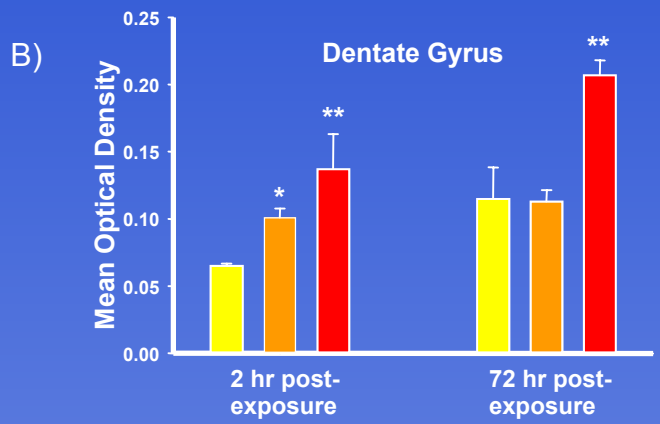
Spatio-Temporal Distribution of BDNF mRNA

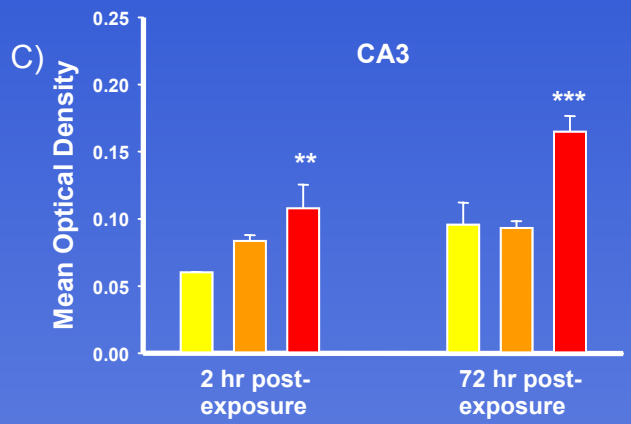


BDNF mRNA expression in the hippocampal region following repeated exposure to low levels of VX







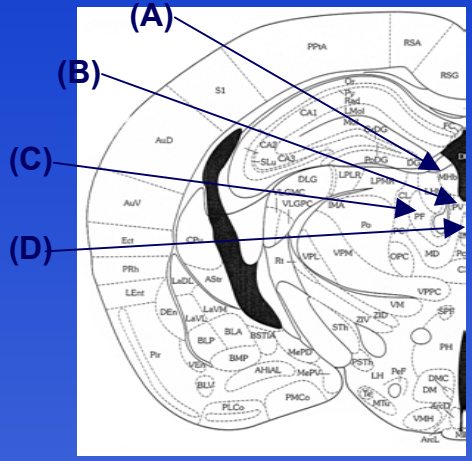


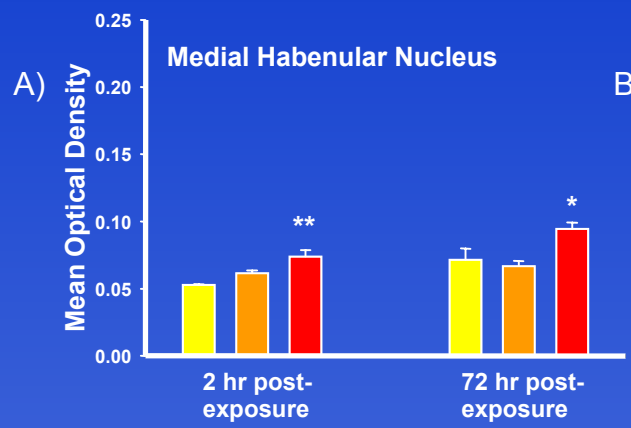
Saline (n = 5)

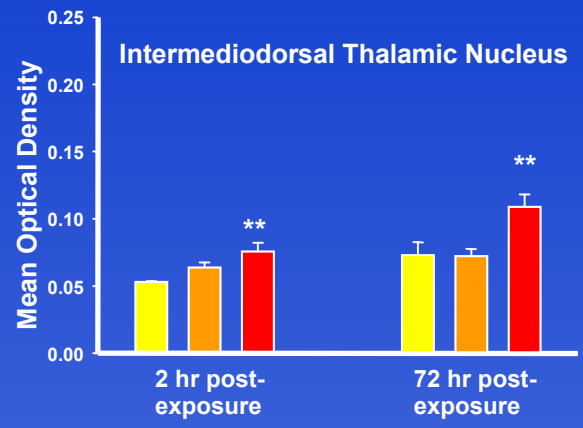
0.2 LD_{50} VX (n = 5)

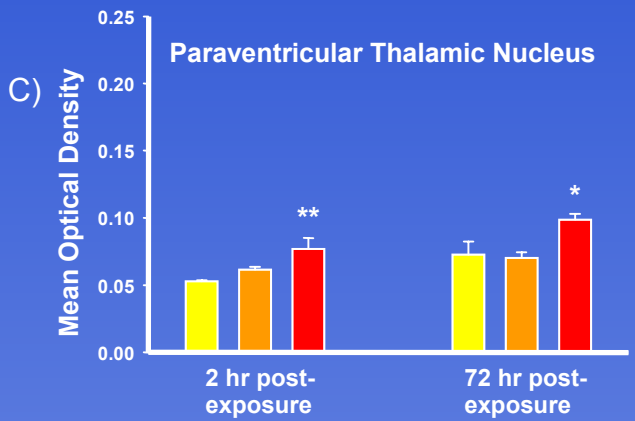
0.4 LD_{50} VX (n = 6)

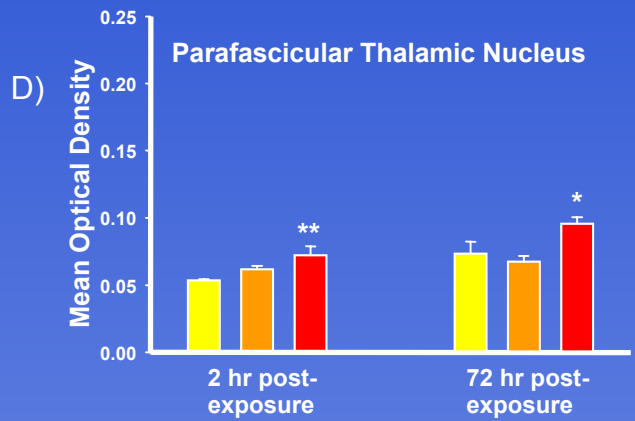
BDNF mRNA expression in the thalamic region following repeated exposure to low levels of VX

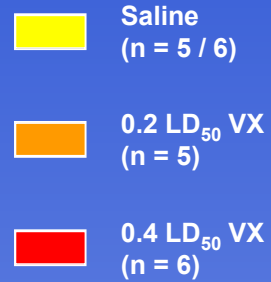




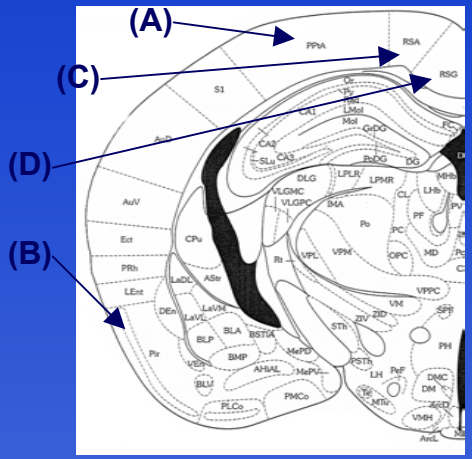


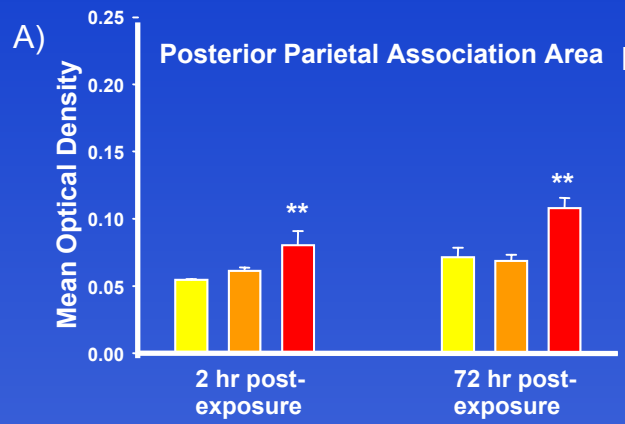


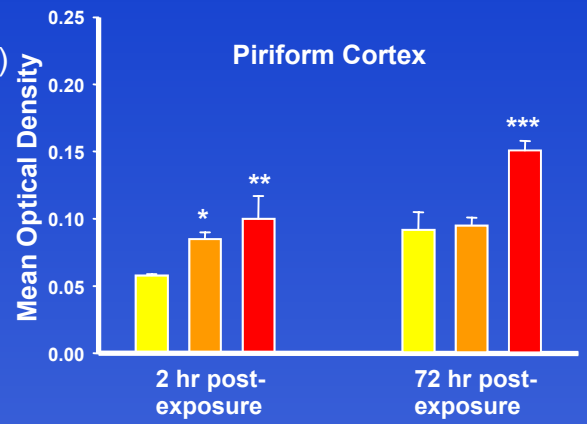


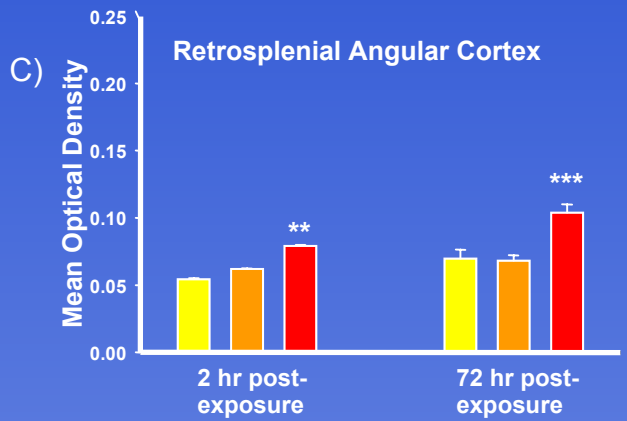


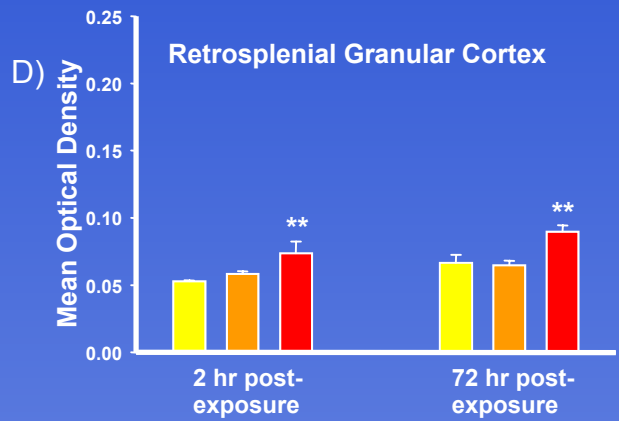
BDNF mRNA expression in the cortical region following repeated exposure to low levels of VX

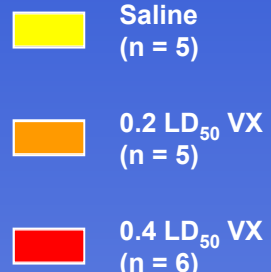




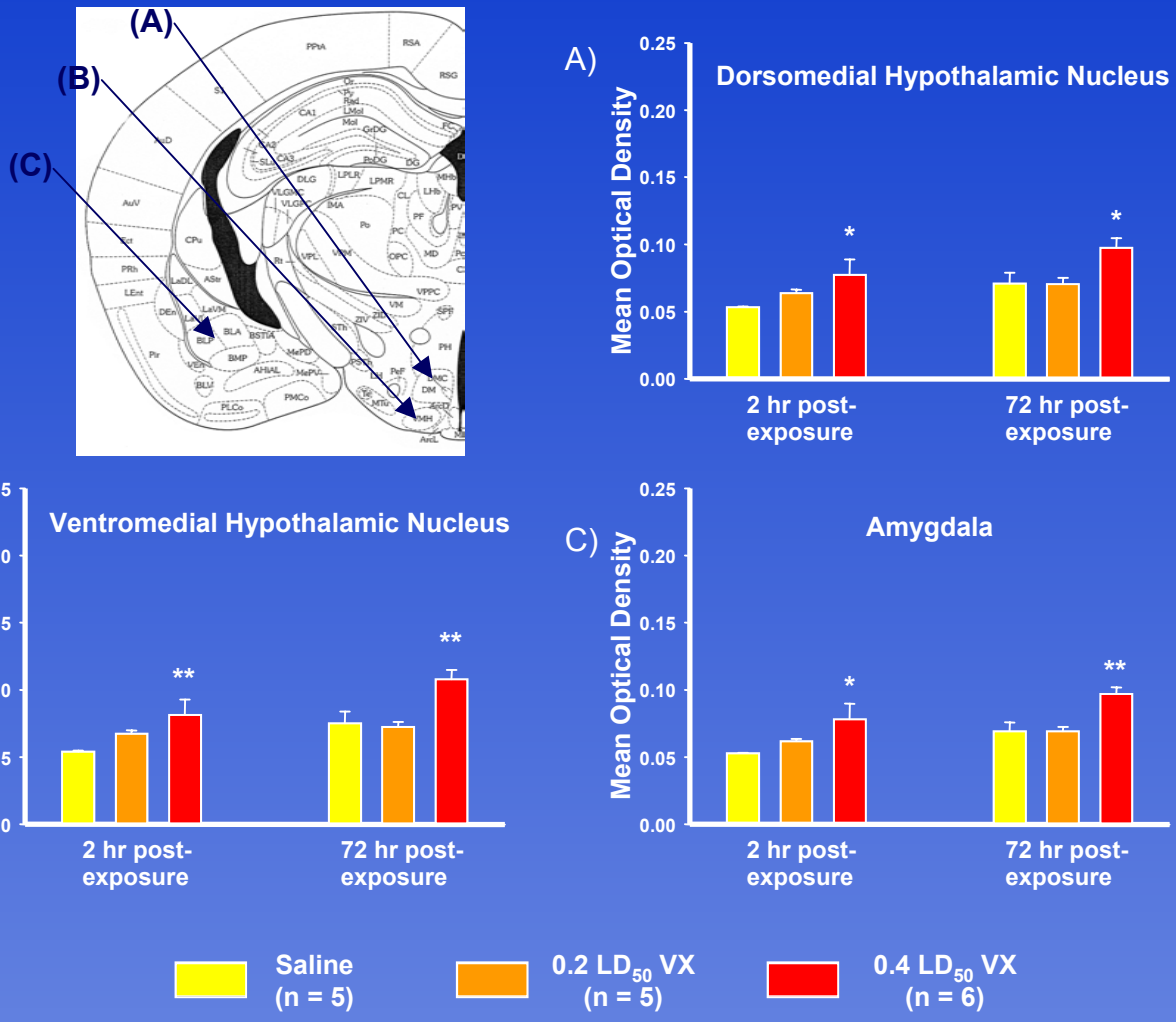








BDNF mRNA expression in the hypothalamic and amygdaloid regions following repeated exposure to low levels of VX



Summary

- We examined the temporal profile of BDNF mRNA expression at 2 hr and 72 hr after repeated injections of either saline, 0.2LD_{50} VX, or 0.4LD_{50} VX using *in situ* hybridization analysis.
- Animals that were injected with 0.4 LD_{50} VX showed elevated levels of BDNF mRNA expression 2 hr and 72 hr later, in areas of the hippocampus, lateral amygdala, cortex, hypothalamus and thalamus.
- In some brain regions, 0.2 LD_{50} VX also increased BDNF expression at 2 hr, but not at 72 hr, post-exposure.

Acknowledgment

- WRAIR
  - Lumley LA
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